Polymer-based Surgically Implantable Haloperidol Delivery Systems and Methods for Their Production and Use

Introduction

This application claims the benefit of priority from
5   U.S. Provisional Patent Application Serial No. 60/242,304,
filed October 20, 2001.

Field of the Invention

The invention relates to surgically implantable drug
delivery systems for the long-term delivery of
10   antipsychotic drugs, and in particular haloperidol. The
surgically implantable drug delivery systems of the present
invention comprise a biodegradable polymer, preferably a
lactide-co-glycolide copolymer, and an antipsychotic drug,
preferably haloperidol, fabricated into an implant via
15   solvent casting and compression molding. As demonstrated
herein, these formulations, when implanted underneath the
skin, release an effective amount of the antipsychotic drug
over a period of months. Also provided in the present
invention are methods for producing and using these
20   surgically implantable drug delivery systems in the
treatment of patients with psychotic disorders such as
schizophrenia.

Background of the Invention

While much research regarding the treatment of
25   schizophrenia has focused on new pharmaceutical compounds,
a major correctable cause for treatment resistance remains
nonadherence with prescribed medication (Fenton et al.
Schizophr. Bull. 1997 23(4):637-51; Kane, J. J. Clin.
Psychopharmacol. 1985 5(3 Suppl):22s-27s). Approximately

50% of patients with schizophrenia and other chronic psychotic conditions are believed to be poorly adherent with prescribed medication (Young et al. Bull. Am. Acad. Psychiatry Law 1986 14(2):105-22). A controlled study to

5 measure adherence with a detectable marker reported that 80% of patients with schizophrenia do not take medications as prescribed (Kapur et al. Schizophr. Res. 1991 6(1):49-53). Studies of relapsed patients have reported only 30% to meet the criteria for good adherence in the months prior

10 to admission (Bergen et al. Aust. N Z J. Psychiatry 1998 32(6):815-22; Razali et al. Acta Psychiatr. Scand. 1995 91(5):331-5).

Therapeutic failure secondary to nonadherence often results in deterioration in social function and more

15 intensive interventions including rehospitalization, such that nonadherence was the most important predictor of rehospitalization in a state hospital population (Casper, E.S. and Regan, J.R. Can. J. Psychiatry 1993 38(10):657-61). Furthermore, length of stay upon rehospitalization is

20 shorter among medication adherent patients and they are less likely to require involuntary admission (McEvoy et al. J. Nerv. Ment. Dis. 1984 172(7):412-6). Without antipsychotic treatment, 75% of patients with schizophrenia relapse within one year of first presentation, compared to

25 15% of treated patients (Ayuso-Gutierrez, J.L and del Rio Vega, J.M. Schizophr. Res. 1997 28(2-3):199-206; Davis, J.M. and Andriukaitis, S. J. Clin. Psychopharmacol. 1986 6(1 Suppl):2S-10S). Discontinuation of antipsychotic medication also has been disclosed to increase risk of

30 relapse approximately 5-fold (Robinson et al. Arch. Gen. Psychiatry 1999 56(3):241-7). Similarly, a study that followed first episode patients for 2 years found relapse rates up to 90% for non-medicated patients (Ram et al. Schizophr. Bull. 1992 18:185-207). Furthermore, good

35 adherence following discharge from the hospital can reduce

recidivism from 73% to 27% within one year (Gaebel, W. and
Pietzeker, A. Pharmacopsychiatry 1985 18(3):235-9).

Consequences of medication nonadherence can extend
beyond the health of the patient. While the majority of
5 people with schizophrenia do not engage in violent
behavior, a subset of patients display aggression during
periods of psychosis (Casper, E.S. and Regan, J.R. Can. J.
Psychiatry 1993 38(10):657-61; Lindqvist, P. and Allebeck,
P. Br. J. Psychiatry 1990 157:345-50; Mitchell, E.W. Med.
10 Sci. Law 1999 39(1):23-30; Tam et al. Psychiatr. Serv. 1996
47(1):86-8).

Efforts to increase medication adherence have
incorporated behavioral and psychoeducational programs,
family interventions and intensive supportive services
15 (Agarwal et al. Int. J. Soc. Psychiatry 1998 44(2):92-106;
Amador, X.F. and Gorman, J.M. Psychiatric. Clin. North Am.
1998 21(1):27-42; Bustillo et al. Harv. Rev. Psychiatry
1999 6(5):229-40; Yong et al. Bull Am. Acad. Psychiatry Law
1986 14(2):105-22).

20   Pharmacological approaches to improved adherence
include improving tolerability and efficacy of
antipsychotic medication (Bustillo et al. Harv. Rev.
Psychiatry 1999 6(5):229-40; Kane, J. Br. J. Psychiatry
Suppl. 1999 37:26-9; Kasper, S. Int. Clin. Psychopharmacol.
25 1998 13 Suppl 3:S71-7; Mauskopf et al. J. Clin. Psychiatry
1999 60(Suppl 19):14-9) through development of new agents
and administration of monthly depot preparations of
existing agents.

Decreased rates of discontinuation were reported for
30 a newer agent, olanzapine, than an older one (haloperidol)
(Tran et al. J. Clin. Psychiatry 1997 Jun 58(5):205-11;
Tran et al. J. Clin. Psychiatry 1997 Jun 58(6):275).
However, newer agents have been reported to have additional
side effects including weight gain, sedation, drooling, Q-T
35 prolongation and agranulocytosis (Campbell et al. Br. J.

Clin. Pharmacol. 1999 47(1):13-22; Wetterling, T. and Mussigbrodt, H.E. J. Clin. Psychopharmacol. 1999 19(4):316-21).

Monthly depot preparations have been reported as an
5 effective means to decrease relapse and rehospitalization (Gerlach, J. Int. Clin. Psychopharmacol. 1995 9 Suppl 5:17-20). Treatment for noncompliant patients with depot formulations (Haldol-decanoate) is much less expensive per year than oral preparations of newer neuroleptics
10 (risperidone; Galzer, W.M. and Ereshefsky, L. J. Clin. Psychiatry 1996 57(8):337-45). However, a 7-year study of depot medication found a significant number of patients fail to comply with monthly injections and discontinuation linked to relapse (Curson et al. Br. J. of Psychiatry 1985
15 146:469-74). Therefore, while depot medication improves adherence initially, many patients still become nonadherent (Weiden et al. Psychiatric Services 1995 46(10):1049-54).

In contrast, a surgically implantable preparation can last for many months, providing patients with symptomatic
20 improvement and possibly delayed disease progression for periods of time never before possible. Additionally, in the event of unacceptable side effects, implants can be removed. This offers a degree of reversibility not presently available with depot formulations. Further,
25 surgically implantable formulations can be employed as a safety net in combination with oral dosing to achieve adjustments as clinically indicated.

Surgically implantable drug delivery systems have been applied in contraception, drug addiction, chemotherapy
30 and pain management. The most widely used, NORPLANT®, (Wyeth Laboratories Inc. Philadelphia, PA) provides 5 years of contraception using levonorgestrel in silicon tubing, allowing for steady state diffusion of active ingredient (Woutersz, T.B. Inter. J. of Fertility 1991 3(51):51-6).
35 Silicon-based delivery systems are ideal for hormonal

delivery due to picogram daily dose requirements. However, this range is not suited for antipsychotics due to slow rates of release.

5   Polymer based delivery systems release milligram range daily dosing required for antipsychotic medications. The polymers used in these systems were initially used in surgical applications (Kulkarni et al. Arch. Surg. 1966 93(5):839-43), but have been modified to integrate medications to form surgically implantable or injectable

10   formulations that release drug over weeks to months. These materials are divided into two categories, namely non-erodible and bioerodible polymers.

       Non-erodible polymers release drug primarily by diffusion while leaving the delivery matrix in place.

15   Non-erodible ethylene vinyl acetate (EVA) is a widely used non-erodible material. Subcutaneous delivery of haloperidol has been demonstrated in rats up to 250 days using EVA (Kohler et al. Neuroscience Letter 1994 170(1):99-102). This preparation delivered steady state

20   levels of haloperidol *in vitro* with physiologic effects on striatal dopamine receptor regulation.

       Bioerodible polymers release medication by erosion of the polymer matrix and diffusion of drug through the remaining polymer matrix. Examples of bioerodible polymers

25   include, but are not limited to, high molecular weight polymers of lactic and glycolic acids, which can be used individually or in lactide-co-glycolide copolymers (PLGA). Advantages of PLGA copolymers include low antigenicity and clearance of breakdown products (lactic and glycolic acid)

30   through the Krebs cycle. These materials have been used in microspheres for injectable depot preparations of chlorpromazine (Gao et al. J. Microencapsul. 1998 15(1):75-83) and haloperidol (Cheng et al. J. Controlled Release 1998 55(2-3):203-12) and are now in clinical use with

35   risperidone (http://www.alkermes.com/index news.html, April

22, 1999). Microspheres are delivered as a suspension, and last approximately 2 weeks.

Various drug delivery devices comprising biodegradable polymers are disclosed generally in, for

5   example, U.S. Patent 5,665,428, U.S. Patent 5,817,343, U.S. Patent 5,871,778, U.S. Patent 5,989,463, U.S. Patent 6,004,573, U.S. Patent 6,117,949, U.S. Patent 6,143,314, and U.S. Patent 6,201,072. These patents each contain an extensive list of possible active agents or therapeutic

10 classes of drugs which are suggested to be deliverable via the drug delivery device.

In addition, U.S. Patent 4,883,666 teaches encapsulation of a compound for treatment of ischemic, metabolic, congenital or degenerative disorders of the

15 central or peripheral nervous system within an implantable biocompatible polymeric device. At col. 13, lines 51-59, it is taught that polymer implants with antipsychotics can be used to treat schizophrenia. Polymer implants used in the examples of the '666 patent all comprised the non-

20 erodible polymer ethylene vinyl acetate (EVA) and dopamine. Further, coating of the implant with a layer of EVA except for one or two holes was required for linear release. Use of bioerodible polymers in implants is suggested at col. 7, line 60 of this patent. However, no examples of implants

25 comprising bioerodible polymers and specific therapeutic agents are taught.

U.S. Patent 5,601,835 teaches similar formulations to those taught in U.S. Patent 4,883,666. However, these formulations are implanted directly into the central

30 nervous system. Again, no examples of implants comprising bioerodible polymers and specific therapeutic agents are taught.

As shown herein, however, not all pharmaceutical agents, and more particularly antipsychotic agents, are

amenable to delivery via systems comprising bioerodible
polymers.

Summary of the Invention

5   An object of the present invention is to provide a
surgically implantable drug delivery system comprising a
biodegradable polymer, preferably a lactide-co-glycolide
copolymer, and an antipsychotic drug, preferably
haloperidol, fabricated into an implant via solvent casting
10 and compression molding.

        Another object of the present invention is to provide
a method of producing surgically implantable drug delivery
systems for haloperidol which comprises dissolving the
haloperidol and a biodegradable polymer, preferably a
15 lactide-co-glycolide copolymer, in an organic solvent;
solvent casting the haloperidol and biodegradable polymer
solution; and molding under compression the solvent cast
solution into a surgical implant.

        Another object of the present invention is to provide
20 a method for treating patients with psychotic conditions
and diseases which comprises surgically implanting into a
patient suffering from a psychotic condition or disease a
surgically implantable drug delivery system comprising a
biodegradable polymer, preferably a lactide-co-glycolide
25 copolymer, and an antipsychotic drug, preferably
haloperidol, which have been fabricated into an implant via
solvent casting and compression molding.

Detailed Description of the Invention

        The present invention relates to a surgically
30 implantable preparation of an antipsychotic agent such as
haloperidol which provides superior treatment outcomes due
to improved medication adherence. The surgically
implantable preparations of the present invention are
designed to last for months to years, providing for

symptomatic improvement as well as possibly delaying
disease progression for periods of time never before
possible. Advantages of administration of antipsychotic
drugs via the surgically implantable delivery systems of

5   the present invention include lower dosing and steady state
serum drug levels, with fewer resultant side effects.
Increased bioavailability with less variation in absorption
and no first pass metabolism also decrease variation in
plasma levels between individuals for a given dose. These

10 factors promote antipsychotic efficacy with reduced drug
exposure and side effects. Additionally, the surgically
implantable drug delivery systems of the present invention
can be used as a low-dose safety net for relapse
prevention, with variable oral dosing of typical or

15 atypical agents for maximum treatment benefit and
supplementation during exacerbations. Finally, in the event
of unacceptable side effects, such as neuroleptic malignant
syndrome, the implant can be removed, thus offering a
degree of reversibility not available with depot

20 formulations.

Surgically implantable delivery systems for
haloperidol have now been created using biodegradable
polymers. In a preferred embodiment, the polymer comprises
polylactide or a copolymer comprising polylactide such as a

25 lactide-co-glycolide copolymer. Preferred biodegradable
polymers comprise about 50 to 100% polylactide and 0 to 50%
polyglycolide. The copolymer and antipsychotic drug,
haloperidol, are fabricated into an implant via solvent
casting and compression molding. In a preferred embodiment

30 of this method, the individual polymers and haloperidol are
dissolved in an organic solvent and solvent cast at a
temperature at which the solvent evaporates for a period of
time which allows for complete drying of the polymer-drug
mixture. For example, for haloperidol and polymers

35 dissolved in acetone, it has been found that solvent

casting at a temperature of about 60°C for approximately 72
hours evaporates all the solvent and results in a
completely dry haloperidol-polymer mixture. Complete
drying can be assessed by weighing the material at the
5   beginning of solvent casting and at the end of the solvent
casting to ensure that all solvent has been evaporated.
Haloperidol concentrations preferably range from about 20%
to about 40% in the delivery system depending upon the
release period. Inclusion of haloperidol in the drug
10   delivery system actually increases the stability of the
drug delivery system. Thus, the higher the concentration
of haloperidol, the more extended the period of release.
This increase in stability does not occur with all drugs.
In fact, other antipsychotic drugs such as thiothixene
15   decreased stability and the period of release of the drug
delivery system when drug concentrations were increased.
Solvent cast material is then compression molded at a
temperature and pressure which allows the polymer-drug
material to flow into the mold. For example, compression
20   molding at 80°C and 25,000 psi (density 1.1±0.05 grams/cc)
has been demonstrated to be suitable for haloperidol
implants of the present invention.

Implants of the present invention were characterized
in vitro for release kinetics. Implants comprising 75:25
25   PLGA with 40% haloperidol resulted in a pattern of release
characterized by an initial phase of slow release (mean =
0.04 mg/day for an average of 21 mg haloperidol load per
implant) from 0 to 28 days. This rate corresponds to
approximately 0.21%/day during the first month. A second
30   phase of more rapid release occurred between 28 and 84 days
(0.27 mg/day per implant), corresponding to approximately
1.29%/day. Implants comprising 85:15 PLGA with 40%
haloperidol displayed a similar pattern of release with a
phase of slow release (mean = 0.05 mg/day for an average of
35   21 mg haloperidol load per implant) from 0 to 56 days,

corresponding to approximately 0.24%/day during the first 2
months. A second phase of more rapid release occurred
between 56 and 140 days (0.23 mg/day per implant),
corresponding to approximately 1.1%/day. Release from a
5   theoretical composite system of 75:25 and 85:15 PLGA, 40%
haloperidol, shows an early phase from 0 to 28 days with an
average of 0.14 mg/day and more rapid release between 28 to
140 days with 0.37 mg/day. Since these values result from
an average drug load of approximately 42 mg, the percent
10   released is 0.33%/day during days 0-28 and 0.88%/day during
days 28-140. The value for the positive control solution
(100 ng/ml) remained stable throughout the period of 140
days. Based on these release rates, a 4 month supply of
haloperidol can be delivered via a 500 mg implant of the
15   present invention comprising 40% haloperidol.

Implants of the present invention were also evaluated
in vivo in rodents for bioactivity. These measures included
behavioral testing with apomorphine stimulated locomotion
in mice, and western blot analyses of D2 receptor
20   expression following implantation in rats. For these
experiments, implant compositions were developed to create
a staged series of implants that release drug for 5 months.
Animals treated with haloperidol implants displayed
increased striatal D2 receptor expression as well as
25   increased apomorphine stimulated locomotion.

In behavioral testing with apomorphine stimulated
locomotion, mice were tested 3 weeks after receiving
implants made of 75:25 PLGA alone or 75:25 PLGA with 20%
haloperidol. Baseline locomotor activity was measured for
30   twenty minutes. Animals with control implants traveled a
mean of 12223 ± 433 cm, while those with haloperidol
containing implants traveled an average of 7664 ± 450 cm.
Thus, mice with haloperidol implants traveled significantly
less distance that the controls ($p < 0.001$). Implants were
35   then removed and all animals were allowed to recover for 48

hours. After recovery, animals received apomorphine 0.5
mg/kg i.p. twenty minutes prior to locomotor testing, which
has been shown to increase locomotor activity in mice
(Ninan, I. and Kulkarni, S.K. Psychopharmacology (Berl)
5 1999 142(2):175-81). After apomorphine challenge, animals
that had control implants traveled a mean of 4721 ± 476 cm,
while those with haloperidol containing implants traveled a
mean of 8531 ± 2536 cm. Therefore, following removal of
implants and exposure to apomorphine, mice that had
10 haloperidol implants traveled more distance than control
mice ($p < 0.02$).

A mean serum level of 26 ng/ml was measured in mice
implanted with haloperidol-PLGA pellets one month following
implantation.

15   Western blots of striatal membranes from all rats
revealed a band at an apparent molecular weight of
approximately 50 kD corresponding to the predicted
molecular weight of the full-length D2 receptor protein
(Expert Protein Analysis System, Swiss Institute of
20 Bioinformatics, http://www.expasy.ch/; Bunzow et al. Nature
1988 336(6201):783-7). Mean optical density of bands were
quantified relative to the corresponding band for
haloperidol-treated rats. Results based on three blots
yielded a mean $\pm$ SD relative to density for haloperidol
25 implant-treated rats of 0.90 ± 0.07 for the 50 kD band.
The mean $\pm$ SD relative density for control rats was $0.64 \pm$
0.02 ($p = 0.0002$, one tail t-test). An additional band of 25
kD was also labeled, likely corresponding to the
intracellular portion of the D2 receptor containing the
30 antigenic peptide against which the antibody was raised.
Quantification of the 25 kD band yielded a relative density
of 0.88 ± 0.12 for haloperidol treated animals and $0.66 \pm$
0.14 for control animals ($p = 0.04$, one tail t-test).

Implants of the present invention have also been
35 placed in monkeys. Monkeys with implants exhibited motor

side effects consistent with haloperidol release within one
day of implantation. Haloperidol serum levels in the
monkeys were assessed at baseline and days 5, 12, 19, 27,
40 and 55 following implantation. Data are depicted in the
5 following Table.

Time (days)	Number of Monkeys	Mean	SD
baseline	3	0.00	0.00
5	3	6.91	0.38
12	3	5.36	1.32
19	3	5.30	0.48
27	2	9.27	6.16
40	2	21.05	8.49
55	2	16.71	1.74

Interestingly, not all pharmaceutical agents nor all
15 antipsychotic agents are amendable to this delivery system.
For example, incorporation of the antipsychotic agent
thiothixene into the implant requires lowering of the
molding temperature by 40°C and causes an acceleration in
degradation of the polymer as opposed to an extension of
20 degradation time as observed with haloperidol. Further,
implant comprising thiothixene degraded at room temperature
without exposure to an aqueous environment within 6 months.
These implants discolored to a yellow shade and liquefied.
In contrast, haloperidol implants of the present invention
25 are stable in storage for periods exceeding one year
without any signs of discoloration or change in
consistency. Incorporation of the anti-depressant
Fluoxetine into this delivery system resulted in an implant
which caused tissue necrosis in 8 out of 8 mice tested. No
30 tissue necrosis was observed in mice with control, Navane
loaded or haloperidol-loaded implants.

Thus, the surgically implantable delivery system of the present invention provides a unique means for the long term delivery of haloperidol.

The delivery systems of the present invention are
5 useful in the treatment of psychotic disorders, particularly disorders such as schizophrenia where patients are oftentimes noncompliant with their medication. The delivery system of the present invention can be surgically implanted into a patient, most preferably under the skin of
10 the patient between the muscle and the dermis, in accordance with well known techniques. Haloperidol released from the bioerodible implant of the present invention maintains its bioactivity and is delivered at steady state concentrations to the patients for periods of
15 five months or more. Implants of the present invention can be used alone or combined with oral supplementation of haloperidol or another antipsychotic drugs for dynamic response to optimum medication levels. Should the patient exhibit unwanted side effects to the haloperidol, the
20 implant can be easily removed.

The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Implant Fabrication
25   Implants were fabricated through solvent casting and compression molding. Two polymers, 75% polylactide with 25% polyglycolide (75:25 PLGA) and 85% polylactide with 15% polyglycolide (85:15 PLGA) are present in a combined system of release during a 5-month period. Each copolymer has a
30 distinctive period of degradation, which is determined by the ratio of lactide to glycolide and the molecular weight of the resulting molecule produced. An additional polymer of 100% polylactide (PLA) was used for *in vivo* testing in rats. All polymers (Alkermes Inc., Cincinnati, OH), has an

inherent viscosity of 0.66-0.8 DL/gram in chloroform and a
molecular weight distribution between 120-140 kD.
Individual polymers and haloperidol (Sigma, St. Louis, MO)
were dissolved in acetone and solvent case at 60°C for 72

5   hours. Solvent cast material was compression molded at 80°C
and 25,000 psi (density 1.1±0.05 grams/cc).

Example 2: *In vitro* assay

Individual implants were placed in 1 liter of
phosphate buffered saline (PBS), pH 7.0 at 37°C in constant

10 motion. Haloperidol amount was measured by GCMS (National
Medical Services, Willow Grove, PA). Each assay included
negative controls of implants made of polymer alone and a
100 ng/ml haloperidol standard to assess stability of
haloperidol in solution over time.

15 ### Example 3: Animals

Implants were tested in rats (Harlan, Indianapolis,
IN) (n=9) and mice (Jackson Labs, Bar Harbor, NM)(n = 16).
All animals were housed in an AAALAC accredited animal
facility at the University of Pennsylvania. Institutional

20 Animal Care and Use Committee (IACUC) approved all
protocols. Animals were maintained with a 12:12 light:dark
cycle with all testing and procedures performed during the
light cycle.

Example 4: Implantation/removal surgery

25   Mice and rats were anesthetized with ketamine/
xylazine (100/10 mg/kg, i.p.). A 1-cm incision was made in
the skin on the dorsal aspect of the animal and an implant
was placed between dermis and muscle. Removal of implants
was performed with identical anesthesia and incision

30 followed by implant retrieval.

Example 5: Behavioral testing

Bioactivity of haloperidol implants was assessed in
mice and rats. Sixteen C57bl/6 mice received implants made
of 75:25 PLGA alone (n=8) or 75:25 with 20% haloperidol

5   (n=8) to assess the effects of implants on locomotion.
Following three weeks of implantation, total distance
traversed was assessed over a twenty-minute period.
Implants were then removed and animals allowed to recover
for 48 hours prior to retesting 20 minutes after

10 apomorphine challenge (0.5 mg/kg i.p.) (Sigma, St Louis,
MO).

Example 6: Western blot

Six Sprague Dawley rats received implants made of PLA
with 30-40% haloperidol. Three rats received implants of

15 PLA alone. Implants remained in all animals for three
months prior to removal. Seventy-two hours after implant
removal, rats were sacrificed and brains rapidly removed,
dissected into four regions (cortex, hippocampus, striatum
and cerebellum) then frozen in liquid nitrogen. Western

20 blots for quantitative analysis of D2 receptor protein were
performed on striatum. Three concentrations of cortex
protein from a single animal (2.5, 5 and 10 µg) were run
with all blots as an internal control to insure intensity
of labeling was within linearity of quantitative software.

25 Only those blots in which the density of samples was within
the linear range of internal standards were used for
analyses. Western blots were performed using polyclonal
antibody, WR-3526, raised against amino acids 272-282 of
the D2 receptor protein (Research and Diagnostic

30 Antibodies, Berkeley, CA). Striata were homogenized in
homogenization buffer (20 mM HEPES, 2 mM EGTA, 1 mM PMSF, 2
µM Aprotinin and 2 mM DTE), followed by a 30-second
sonication. Samples were centrifuged 100,000 g for 1 hour
at 4°C. Pellets were resuspended and solubilized in

homogenization buffer containing 0.1% Triton X-100.
Proteins were extracted on ice for 45 minutes with
occasional agitation. After extraction, proteins were
centrifuged at 30,000 g for 30 minutes at 4°C. Protein
5   samples were prepared with 25% 4x NuPAGE sample buffer plus
10% reducing agent (Invitrogen) and heat shocked at 70°C for
10 minutes. Samples were separated on a 10% precasted
mini-gel at 200 volts for 50 minutes. Proteins were
transferred to PVDF at 30 volts for 1 hour. Blots were
10 blocked with 5% milk TBS (20 mM Tris, pH=7.5, 0.5 mM NaCl),
then washed for 15 minutes. Blots were then incubated
overnight with anti-D2-receptor antibody, washed with TBS,
and incubated with goat-anti-rabbit horseradish peroxidase
conjugate (BioRad, 1:4800) for 1 hour. Blots were then
15 incubated with chemiluminescent substrate (Pierce) for 1
minute, wrapped with plastic and exposed to
autoradiographic film.

Example 7: Quantification

The intensity of each band was quantified using a
20 densitometer model 7100 and quantitative analysis software
(Bio Rad, Hercules, CA) and expressed as a ratio to the
corresponding band in rat 1 to yield a ratio of intensity
(rat 1 = ratio of 1). All samples were processed
simultaneously on a single blot to allow for quantitative
25 comparisons between conditions.